Effect of synchronization of follicle-wave emergence with estradiol and progesterone and superstimulation with follicle-stimulating hormone on milk estrogen concentrations in dairy cattle

Lucilene B. de Souza, Raynald Dupras, Louis Mills, Younès Chorfi, Christopher A. Price

Abstract

Very little is known about the effects of hormonal synchronization of follicle waves and superovulation on the estrogen content of a cow's milk. The objective of this study was to determine the effect in dairy cows of synchronization with estradiol-17β (E2) and progesterone (P4) on milk E2 concentrations and to compare these levels with those achieved during superstimulation for 4 d with porcine follicle-stimulating hormone (FSH). The milk E2 concentrations were raised significantly above pretreatment levels ($P < 0.05$) for 2 d after synchronization, the mean peak being 40.2 ± 18.5 (standard error) pg/mL and the pretreatment mean 1.5 ± 0.5 pg/mL. The mean peak E2 concentration during ovarian stimulation was 4.4 ± 0.7 pg/mL. The mean E2 concentration was significantly higher ($P < 0.05$) after synchronization than during superstimulation for the 1st milking after synchronization but not subsequent milkings. The milk estrone concentrations were above pretreatment levels for 1 d after synchronization and were not different from those observed during superstimulation.

Résumé

Il y a peu d'information sur l'effet de la synchronisation hormonale de la vague folliculaire et de la surovulation sur la quantité d'œstrogène dans le lait de la vache. L'objectif de cette étude était de déterminer l'effet de la synchronisation avec le 17β-estradiol (E2) et la progestérone (P4) sur les concentrations de E2 dans le lait et de les comparer avec celles mesurées après stimulation avec la hormone folliculostimulante (FSH). Des vaches laitières ont été synchronisées avec E2 et P4 et surstimulées pendant 4 jours avec la FSH porcine. Le protocole de synchronisation a augmenté significativement ($P < 0,05$*) les concentrations E2 dans le lait par rapport aux niveaux du prétraitement pendant les 2 jours suivant la synchronisation (concentrations maximales de 40,2 ± 18,5 pg/mL contre 1,5 ± 0,5 pg/mL prétraitement). Ces concentrations étaient significativement plus élevées (*$P < 0,05$*) que celles observées pendant la surstimulation (4,4 ± 0,7 pg/mL) pour la 1ère traite après la synchronisation. Les concentrations de l'estrone dans le lait étaient plus élevées que celles du prétraitement pour 1 jour après synchronisation, et elles n'étaient pas différentes des niveaux observés pendant la surstimulation.*

(Traduit par les auteurs)

Synchronization of follicle-wave emergence is an important tool for effective superovulation in ruminants, as it allows fixed-time artificial insemination without the need for estrus detection. One of the more effective synchronization methods involves the combined administration of estradiol-17β (E2) and progesterone (P4), which causes regression of the dominant follicle and then emergence of a new follicle wave (1); superstimulation is typically started on the day of wave emergence. This method is more practical than follicle ablation and gives more consistent results than traditional protocols involving gonadotropin-releasing hormone and luteinizing hormone (GnRH/LH) (2). In a summary of several studies Bó et al (3) reported the emergence of a new follicle wave in 94% of cattle injected with 5 mg of estradiol, irrespective of the stage of the estrous cycle; in comparison, the rates were 40% to 80% for reviewed GnRH/LH protocols (2).

The use of E2 to synchronize follicle-wave emergence in food-producing animals is no longer permitted in Europe and several other countries owing to concerns about steroid residues in food (4). Nevertheless, the practice remains common in some countries in beef and dairy cattle (5,6) and in other species, such as lactating llamas (7). The use in North America of E2 to synchronize follicle-wave emergence and induce ovulation is still being reported in research papers (8), and in some countries, including Canada, E2 esters are permitted for the treatment of anestrus and cysts in cattle. Further, a recent publication states that E2 valerate is approved by the US Food and Drug Administration for use in nonlactating cows (9).

Estimates of estrogen concentrations in commercial cow milk vary from 1 to 6 pg/mL for E2 and 20 to 150 pg/mL for estrone (E1) (10–12) and are argued to be too low to affect human health (12). Very little is known about the impact of reproductive technologies in ruminants on the estrogen content of milk. It is well known that superovulation results in elevated plasma E2 concentrations (13–15), and 1 study found about a 3-fold increase in E2 content in defatted milk from cows superovulated with porcine follicle-stimulating

Centre de recherche en reproduction animale, Faculté de médecine vétérinaire, Université de Montréal, 3200, rue Sicotte, Saint-Hyacinthe, Québec J2S 7C6 (de Souza, Chorfi, Price); Medi-Vet Inc, 167, rue Notre-Dame, Notre-Dame-du-Bon-Conseil, Québec J0C 1A0 (Dupras, Mills).
Address all correspondence to Dr. Christopher A. Price; telephone: 450-773-8521; fax: 450-778-8103; e-mail: christopher.price@umontreal.ca
Received November 15, 2011. Accepted March 7, 2012.

hormone (FSH) (16). Plasma E1 concentrations increased during the periovulatory period in cattle stimulated with equine chorionic gonadotropin, but a direct comparison with nonstimulated animals was not made (14). The objective of the present study was to determine the impact of an E2–P4 synchronization protocol on milk estrogen concentrations in dairy cattle and to compare these concentrations with those generated by a standard superovulation protocol.

The study was conducted on a commercial dairy farm in Quebec and was approved by the Animal Use and Care Committee of the Faculty of Veterinary Medicine, at St. Hyacinthe. Six cows [mean age 5.5 ± 0.8 (standard error) y, body weight 671 ± 9 kg, parity 2 to 4, time in lactation 100 ± 8 d, milk production 37.9 ± 0.8 L/d] were selected at random to undergo a synchronization/superovulation protocol. On the afternoon of day 0 of the protocol the cows were given an intravaginal P4 implant (CIDR; Pfizer Canada, Kirkland, Quebec) and 4.5 mg of E2 in sesame oil (Gentès & Bolduc, St. Hyacinthe, Quebec) by intramuscular (IM) injection. Superstimulation was started on the evening of day 4; the total dose of 380 mg of NIH FSH P1 (Folltropin-V; Bioniche Animal Health, Belleville, Ontario) was given as 9 IM injections of decreasing dose at 12-h intervals. Cloprostenol, 2 mg IM (Estrumate; Merck Animal Health, Kirkland, Quebec) was given in the morning and evening of day 7, the CIDR was removed on day 8, and 150 μg of GnRH (Cystorelin; Mérial Canada, Baie d'Urfé, Quebec) was given as 2 injections 12 h apart on day 9.

The animals were milked in a unit equipped with an in-line monitoring device (Metatron, GEA WestfaliaSurge Canada, Mississauga, Ontario), and milk was collected continuously during each milking to obtain a homogeneous sample at each time point for each cow. Samples were collected immediately before E2 and CIDR administration and twice daily thereafter until day 10. Samples were frozen at −20°C until assayed.

Whole-milk samples were homogenized with a Brinkmann Polytron PT3000 (Brinkmann Intruments, Westbury, New York at time of purchase, which is now owned by Metrohm USA, Riverview, Florida, USA) and assayed after solvent extraction. Each 2-mL sample was spiked with tritiated E2 or E1 for estimating extraction efficiency and were extracted twice with 6 mL of ethyl acetate:*n*-hexane 1:1 in borosilicate glass tubes. The extract was dried under nitrogen, dissolved in 2 mL of methanol, and then incubated for 1 h at 50°C. The samples were then centrifuged at 400 × *g* for 30 min at 4°C, and the supernatant was transferred to clean tubes and dried under nitrogen. The residue was reconstituted in assay buffer (0.1% gelatin in phosphate-buffered saline). The extraction efficiencies were approximately 68% and 43% for E2 and E1, respectively.

Sample extracts were measured in duplicate with enzyme-linked immunoassay kits (SLV-4188 and EIA-4174, for E2 and E1, respectively; DRG Diagnostics, Marburg, Germany) and values corrected for extraction losses. According to the manufacturer the E2 assay had 0.2% cross-reactivity with E1 and the E1 assay had less than 0.1% cross-reactivity with E2; cross-reactivity with other steroids was less than 0.1%. The sensitivities of the assay curves were 1 and 15 pg/mL for E2 and E1, respectively (the lowest standard in each kit), which was equivalent to 0.3 and 6 pg/mL of milk, respectively. The intra- and interassay coefficients of variation for both assays were less than 5%.

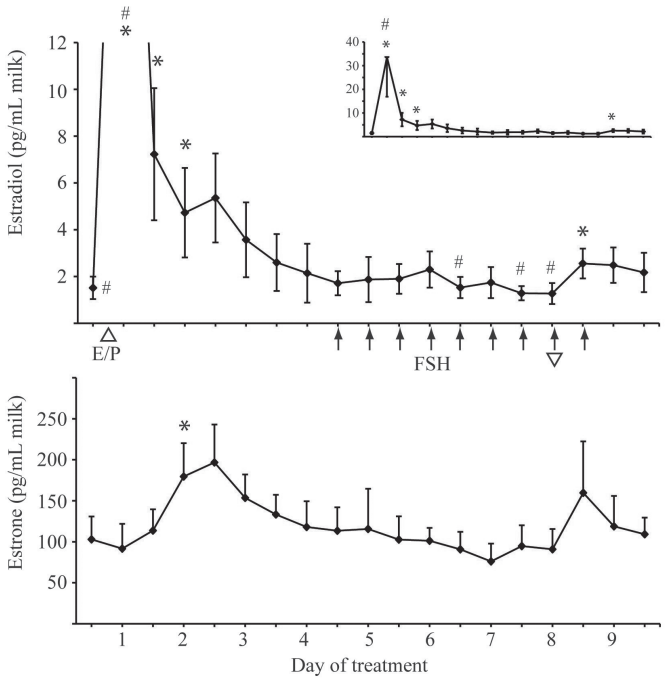

Figure 1. Concentrations of estradiol (E2) and estrone in milk after follicle-wave synchronization with E2 and progesterone (P4) as well as ovarian stimulation with follicle-stimulating hormone (FSH). The E2 concentration in the 1st milk sample is not shown in the main figure but is presented in the inset. Data are means ± standard error for 6 cows. * — significantly different ($P < 0.05$) from the mean for the pretreatment samples; # — significantly different ($P < 0.05$) from the mean for the day-1 samples (not shown for all samples on the inset); E/P — E2 injection and intravaginal implantation of CIDR; arrows — FSH injections; P — CIDR removal.

Preliminary experiments demonstrated that when serial dilutions of milk were extracted as described, the resulting extracts (not corrected for extraction volume) were parallel with the standard curve. Mean recoveries of added steroid from milk were 102% for E2 and 94% for E1.

The data were not normally distributed and were log-transformed for repeated-measures analysis of variance with JMP software (SAS Institute, Cary, North Carolina, USA). Pretreatment samples were compared with samples collected at all other time points to determine treatment effects. The data are presented as means ± standard error. Peak superovulatory milk E2 concentration is defined as the maximum concentration reached for each individual (irrespective of time of milking) during FSH treatment, and peak synchronization milk E2 concentration is defined as the maximum concentration reached within 5 d of E2 administration.

Average concentrations of E2 and E1 before synchronization were 1.5 ± 0.5 and 103 ± 28 pg/mL, respectively. The values for E2 are consistent with those reported by others (10,12), and the values for E1 are similar to those reported by Hartmann et al (17) but higher than those reported more recently (10,12).

The mean milk E2 and E1 profiles are shown in Figure 1. The main objective of the study was to determine the effect of an E2–P4 protocol for follicle-wave synchronization on milk E2 content. As expected, this protocol had an immediate and significant impact on milk E2 concentration, the concentrations being significantly

($P < 0.05$) above pretreatment levels for 3 successive milkings after E2 injection. Peak E2 levels occurred at the 1st milking for 4 animals and at the 2nd milking for 2 animals; the mean peak synchronization E2 concentration was 40.2 ± 18.5 pg/mL.

The mean E2 concentrations during ovarian stimulation were significantly higher ($P < 0.05$) than the pretreatment levels (compare day 0.5 with day 8.5 in Figure 1), and the mean peak E2 concentration was 4.4 ± 0.7 pg/mL, 3-fold higher than the pretreatment levels. These data are entirely consistent with those in the only other report in the literature on measurement of the effect of superovulatory protocols on milk estrogen content, in which an increase of 3 to 4 times in the E2 concentration in defatted milk was described (16).

To compare the effects of synchronization and superstimulatory treatments, the values on day 8.5 were compared with the values after administration of E2–P4; only the 1st milk sample (collected on day 1) had significantly higher ($P < 0.01$) E2 concentrations than the day-8.5 sample. The pretreatment milk and the samples collected on days 6.5, 7.5, and 8 contained significantly less E2 ($P < 0.05$) than the sample collected on day 8.5. It should be noted that the dose of estradiol used is within the range previously reported (18), although lower doses may be equally effective (18) and would likely result in lower milk E2 concentrations than those reported here.

The milk concentration of E1 was less affected by treatment than were the milk concentrations of E2. Synchronization doubled the E1 concentration ($P < 0.05$) on day 2 after E2–P4 administration, and FSH treatment caused a nonsignificant increase of 50% above pretreatment levels. The increase in E1 level during FSH treatment is likely caused by the increased number of follicles and therefore steroidogenic activity. The increase in E1 level after E2 administration is most likely due to peripheral metabolism of E2, as E1 is not synthesized by mammary tissue (19), and previous studies have demonstrated that 14% to 30% of exogenous E2 is metabolized to E1 (20,21).

In conclusion, the present study has demonstrated that milk estrogen content after an E2-based synchronization protocol is not elevated significantly above levels seen during routine superovulatory protocols, except for the 1st day after E2 administration.

Acknowledgments

We thank Bioniche Animal Health, Belleville, Ontario, for donating the Folltropin-V, Noureddine Jinane for collecting the milk samples, and farm staff for care of the animals. This work was supported by Natural Sciences and Engineering Research Council of Canada (Engage program).

References

1. Bó GA, Baruselli PS, Moreno D, et al. The control of follicular wave development for self-appointed embryo transfer programs in cattle. Theriogenology 2002;57:53–72.
2. Bó GA, Guerrero DC, Tribulo A, et al. New approaches to superovulation in the cow. Reprod Fertil Dev 2010;22:106–112.
3. Bó GA, Adams GP, Caccia M, et al. Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Anim Reprod Sci 1995;39:193–204.
4. Lane EA, Austin EJ, Crowe MA. Oestrous synchronisation in cattle — current options following the EU regulations restricting use of oestrogenic compounds in food-producing animals: A review. Anim Reprod Sci 2008;109:1–16.
5. Sá Filho MF, Santos JE, Ferreira RM, et al. Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to estradiol/progesterone-based timed insemination protocols. Theriogenology 2011;76:455–463.
6. Vasconcelos JL, Jardina DT, Sá Filho OG, et al. Comparison of progesterone-based protocols with gonadotropin-releasing hormone or estradiol benzoate for timed artificial insemination or embryo transfer in lactating dairy cows. Theriogenology 2011;75:1153–1160.
7. Aller JF, Cancino AK, Rebuffi GE, et al. Effect of estradiol benzoate used at the start of a progestagen treatment on superovulatory response and embryo yield in lactating and non-lactating llamas. Anim Reprod Sci 2011;119:322–328.
8. Souza AH, Silva EPB, Cunha AP, et al. Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. Theriogenology 2011;75:722–733.
9. Modric T, Momcilovic D, Gwin WE, et al. Hormonal and antimicrobial therapy in theriogenology practice: Currently approved drugs in the USA and possible future directions. Theriogenology 2011;76:393–408.
10. Courant F, Antignac JP, Laille J, et al. Exposure assessment of prepubertal children to steroid endocrine disruptors. 2. Determination of steroid hormones in milk, egg, and meat samples. J Agric Food Chem 2008;56:3176–3184.
11. Malekinejad H, Scherpenisse P, Bergwerff AA. Naturally occurring estrogens in processed milk and in raw milk (from gestated cows). J Agric Food Chem 2006;54:9785–9791.
12. Pape-Zambito DA, Roberts RF, Kensinger RS. Estrone and 17β-estradiol concentrations in pasteurized–homogenized milk and commercial dairy products. J Dairy Sci 2010;93:2533–2540.
13. Kaneko H, Watanabe G, Taya K, et al. Changes in peripheral levels of bioactive and immunoreactive inhibin, estradiol-17 beta, progesterone, luteinizing hormone, and follicle-stimulating hormone associated with follicular development in cows induced to superovulate with equine chorionic gonadotropin. Biol Reprod 1992;47:76–82.
14. Saumande J, Lopez-Sebastian A. Changes in the plasma concentrations of free and conjugated oestrogens in heifers after treatment to induce superovulation and the relationship with number of ovulations. J Reprod Fertil 1982;66:411–416.
15. Soumano K, Silversides DW, Doizé F, et al. Follicular 3β-hydroxysteroid dehydrogenase and cytochromes P450 17α-hydroxylase and aromatase messenger ribonucleic acids in cattle undergoing superovulation. Biol Reprod 1996;55:1419–1426.
16. Saumande J, Batra SK. Superovulation in the cow: Comparison of oestradiol-17β and progesterone patterns in plasma and milk of cows induced to superovulate; relationships with ovarian responses. J Endocrinol 1985;107:259–264.
17. Hartmann S, Lacorn M, Steinhart H. Natural occurrence of steroid hormones in food. Food Chemistry 1998;62:7–20.

18. Martínez MF, Kastelic JP, Bó GA, et al. Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. Anim Reprod Sci 2005;86:37–52.
19. Janowski T, Zduńczyk S, Małecki-Tepicht J, et al. Mammary secretion of oestrogens in the cow. Domest Anim Endocrinol 2002;23:125–137.
20. Dunn TG, Kaltenbach CC, Koritnik DR, et al. Metabolites of estradiol-17β and estradiol-17β-3-benzoate in bovine tissues. J Anim Sci 1977;45:659–673.
21. Ivie GW, Christopher RJ, Munger CE, et al. Fate and residues of [4–14C] estradiol-17β after intramuscular injection into Holstein steer calves. J Anim Sci 1986;62:681–690.